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Follicular wave emergence in Santa Inês ewes subjected to long-term, progesterone-based estrous synchronization protocols at different times of the year

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ABSTRACT

This study was conducted to document the pattern of antral follicular wave emergence throughout the 14-day, progesterone (P_4)-based estrous synchronization protocol in ewes that were maintained in subtropical conditions, during the period of increasing day lengths (ID), decreasing day lengths (DD), and the transitional period (TP). In addition, the influence of ovarian status (i.e., size of ovarian antral follicles and the presence of corpora lutea) at the outset of P_4 treatment on ensuing ovarian follicular wave development was examined. Sexually mature Santa Inês ewes ($n = 70$) were subjected to one of the two estrous synchronization protocols in the three seasons. On Day 0, the ewes received an i.m. injection of prostaglandin $F_{2\alpha}$ and an intravaginal P_4-releasing device that remained in place for 14 days (G-1CIDR) or was replaced on Day 7 (G-2CIDR). Daily ultrasonography of ovaries was conducted from Days 0 to 15. Mean (±SEM) numbers of follicular waves per ewe were 3.7 ± 0.1 and 3.6 ± 0.1 for G-1CIDR and G-2CIDR ($P > 0.05$). The number of emerging follicular waves was greater ($P < 0.05$) during the ID period than during the TP and DD periods (4.0 ± 0.1, 3.4 ± 0.1 and 3.6 ± 0.1, respectively). The presence of medium-sized antral follicles (4.0 to 5.75 mm) in the absence of corpora lutea at the time of CIDR insertion tended to advance follicular wave emergence. Although the long-term P_4 treatment was not originally designed to synchronize follicular waves, there was a distinctive pattern of follicular wave dynamics during the period of application of CIDRs that was affected mainly by the number of emerging follicular waves and ovarian status at CIDR insertion.

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1. Introduction

Multiple ovulation and embryo transfer (MOET) programs in sheep have successfully been implemented in many parts of the world (Menchaca et al., 2010). At present, the imperfections of superovulatory protocols are seemingly the major reason for the great variability in the ovulatory responses and embryo yields; this apparent drawback being arguably one of the primary factors limiting the widespread application of MOET in sheep (Oliveira, 2011). A typical superovulatory treatment regimen begins with the estrous synchronization using a 12 to 14 day treatment with progestagen (Fonseca et al., 2007). In spite of the unquestionable benefits of the hormonally induced estrous synchronization (Sir and Bartlewski, 2009), there are potential undesirable effects of the prolonged progestagen treatment in ewes. During the use of progesterone (P_4)-releasing intravaginal devices, circulating concentrations of the steroid are not sustained and there is a rapid decrease in serum P_4 concentrations before the end of the long-term treatment (between days 10 and 14; Letelier et al., 2009). Varying P_4 concentrations may alter the pattern of antral follicular emergence and growth, which in turn may influence ovulation, fertilization processes and subsequent embryo development (González-Bulnes et al., 2005). One of the strategies employed to circumnavigate these effects of the 14-day estrous synchronization protocols on antral follicular kinetics is to replace the source of P_4, approximately half-way through the period of treatment, to avoid the occurrence of less than normal mid-luteal phase concentrations of the hormone (González-Bulnes et al., 2002; Oliveira et al., 2012).

The number and size of ovarian antral follicles at the beginning of the super-ovulatory treatment is another factor that can impinge on the ovarian responses in donor ewes. With the use of traditional super-ovulatory protocols, 70% to 85% of donors have large antral follicles on the first day of follicle-stimulating hormone (FSH) administration (Menchaca et al., 2007). The presence of large follicles may alter the timing of the preovulatory luteinizing hormone surge (D'Occhio et al., 1999), ovulatory response as well as embryo yields and quality (Veiga-Lopez et al., 2006; López-Alonso et al., 2005). Alternatively, super-ovulatory treatments initiated at or around the time of follicular wave emergence result in greater and less variable ovulatory responses and embryo yields compared with the treatments begun in the presence of a large, growing antral follicle(s) from the previous wave(s) (Menchaca et al., 2007).

There has been no earlier study of antral follicular dynamics during the long-term CIDR treatments, and no report of photoperiodic and ovarian influences on follicular wave emergence during P_4-based, estrous synchronization in ewes maintained in subtropical conditions. The Santa Inês is a meat breed of wool-less sheep originating in Brazil (Rajab et al., 1992). It was derived from crosses of the Morada Nova, Bergamasca, and native coarse-wool sheep, and is very well adapted to tropical and subtropical climates. There is a paucity of information on the reproductive characteristics of Santa Inês sheep; however, it is possible that some Santa Inês ewes exhibit some degree of sensitivity to photoperiodic changes and undergo anestrus in southeastern Brazil (Balaro et al., 2014).

Therefore, the present study was undertaken to describe and compare the antral follicular wave kinetics during the 14-day period of applying estrous synchronization protocols, with or without CIDR replacement, in ultrasonographically monitored Santa Inês ewes at three distinctive times of the year (a period of decreasing and increasing day lengths as well as the transitional period characterized by relatively stable, long photoperiods, as described by López-Alonso et al., 2005). In addition, the influence of ovarian status (e.g., the size of antral follicles and/or presence of corpora lutea) was examined at the outset of P_4 treatment on the ensuing pattern of follicle wave emergence.

2. Material and methods

2.1. Location, animals and experimental procedures

The present study was conducted in the College of Agricultural and Veterinary Sciences (FCAV) situated in the municipality of Jaboticabal (latitude: 21°15'18"S, longitude 48°19'19"W), São Paulo State, Brazil. In this particular region, there exist three distinctive periods of the year characterized by varying duration of the day length: i. a period from the beginning of winter to the beginning of summer, or between July and November – a period of increasing day lengths; ii. from late summer to early winter, or between March and June – a period of decreasing day lengths; and iii. transitional period (during the summer or between December and February) with relatively consistent long days (López-Alonso et al., 2005).

All experimental procedures were compliant with the guidelines on the Ethics and Animal Welfare, and had been approved by the animal care committee of the College of Agricultural and Veterinary Sciences (FCAV), São Paulo State University "Júlio de Mesquita Filho" (protocol no. 003261-08). Sexually mature ($n = 70$) and clinically healthy Santa Inês ewes (aged between 2 and 3 years, mean ($\pm$SEM) body weight of 41.4 ± 2.9 kg) were used in the present study. The ewes were subjected to one of two synchronization protocols at three different times of the year (2×3 factorial design; a period of increasing day lengths (ID): G-1CIDR, $n = 12$ and G-2CIDR, $n = 11$; transitional period (TP): G-1CIDR, $n = 12$ and G-2CIDR, $n = 12$; and a period of decreasing day lengths (DD): G-1CIDR, $n = 11$ and G-2CIDR, $n = 12$). Different animals were used during each reproductive phase. Animals were maintained in paddocks with easy access to sheds and were exposed to natural photoperiods and ambient temperatures. Animals were fed corn silage and nutrient balanced feed (200 g/ewe/day) twice daily, and had ad libitum access to water and mineral salt licks.

On Day 0 (random day of the estrous cycle or anovulatory period), all animals were fitted with an intravaginal P_4-releasing device (CIDR™; Pfizer, Austin, New Zealand), which was maintained in place for 14 days (G-1CIDR), and received an i.m. injection of 10 mg of prostaglandin $F_{2\alpha}$ (Lutalyse™; Pfizer, Austin, New Zealand). In the three subsets of animals, the CIDR's were replaced on Day 7

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